Amendment of claim

Amending claims as agreed upon during the phone interview on November 23, 2004, as follow:

Claim 4. A quantitative method for molecular diagnosis of spinal muscular atrophy (SMA) comprising:

- obtaining a sample from a human suspected of having SMA containing mRNA of survival motor neuron (SMN-mRNA) and the mRNA of human elongation factor 1-alpha (HUMEF1AB-mRNA)
- reverse transcribing the mRNA using primers consisting of SEQ ID NO:1 for the synthesis of cDNA from SMN-mRNA giving SMN-cDNA and consisting of SEQ ID NO:2 for the synthesis of cDNA from HUMEF1AB-mRNA giving HUMEF1AB-cDNA;
- amplifying the SMN-cDNA by PCR using primers consisting of SEQ ID NO:3 and SEQ ID NO:4 and the HUMEF1AB-cDNA by PCR using primers consisting of SEQ ID NO:5 and SEQ ID NO:6
- immobilizing of the PCR products on a nylon membrane
- hybridizing the immobilized PCR products with radioactive ^{32}p-dCTP labeled nucleotide probes wherein the probes are generated by PCR amplification of nucleic acids consisting of SEQ ID NO:7 and 8 for exon 7; nucleic acids consisting of SEQ ID NO:9 and SEQ ID NO:4 for exon 8 and, nucleic acids consisting of SEQ ID NO:5 and SEQ ID NO:6 for HUMEF1AB,
- measuring SMN-mRNA by means of BioImager device using the radioactive ^{32}p-dCTP labeled nucleotide probes by detecting the hybridized probes using

autoradiography and quantifying the amount of SMN-mRNA by means of a BioImager device

- wherein the quantification of between 9 and 27 PSL/mm2 for exon 7 and between 6 and 15 PSL/mm2 for exon 8 is indicative of SMA disease.

Claim 5. A quantitative method for molecular diagnosis of spinal muscular atrophy (SMA) comprising:

- obtaining a sample from a human suspected of having SMA containing mRNA of survival motor neuron (SMN-mRNA) and the mRNA of human elongation factor 1-alpha (HUMEF1AB-mRNA)
- reverse transcribing the mRNA using primers consisting of SEQ ID NO:1 for the synthesis of cDNA from SMN-mRNA giving SMN-cDNA and consisting of SEQ ID NO:2 for the synthesis of cDNA from HUMEF1AB-mRNA giving HUMEF1AB-cDNA;
- amplifying the SMN-cDNA by PCR using primers consisting of SEQ ID NO:3 and SEQ ID NO:4 and the HUMEF1AB-cDNA by PCR using primers consisting of SEQ ID NO:5 and SEQ ID NO:6 in the presence of digoxigenin-11-dUTP
- hybridizing the obtained PCR products with the biotin-11-dCTP labeled nucleotide probes wherein the probes are generated by PCR amplification of nucleic acids consisting of SEQ ID NO:7 and 8 for exon 7; nucleic acids consisting of SEQ ID NO:9 and SEQ ID NO:4 for exon 8 and, nucleic acids consisting of SEQ ID NO:5 and SEQ ID NO:6 for HUMEF1AB,

- immobilizing the hybridized products on streptavidin coated polystyrene microtitration plates
- adding the peroxidase-conjugated anti-digoxigenin antibodies
- adding the peroxidase substrates consisting of H_2O_2 and chromogene (tetramethyl benzidine)
- adding H_2SO_4 to stop the reaction
- measuring SMN-mRNA by means of microplate reader in an ELISA procedure using the biotin-11-dCTP labeled nucleotide probes immobilized on streptavidin polystyrene microtitration plates by detecting the hybridized probes using ELISA and quantifying the amount of SMN-mRNA by determining the optical density (OD)
- wherein an OD of between 0.11 and 0.19 for exon 7 and an OD of between 0.12 and 0.16 for exon 8 is indicative of SMA disease.